# Modelling of activated sludge acclimisation to a non-ionic surfactant

**G. Carvalho*, I. Nopens**, J.M. Novais*, P.A. Vanrolleghem** and H.M. Pinheiro***

*Centro de Engenharia Biológica e Quìmica, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa Codex, Portugal

**BIOMATH Department, Ghent University, Coupure Links 653, B-9000 Gent, Belgium

**Abstract** A model is proposed to describe activated sludge acclimatisation to a non-ionic surfactant. The model was calibrated automatically, using WEST, a specific software environment for wastewater treatment model building, simulation and parameter estimation. The assays have been performed in a sequencing-batch reactor (SBR), using a non-ionic surfactant as sole carbon source and non-acclimatised sludge. The best fitting model was based on the assumption of three sequentially degraded COD fractions, where the second fraction is a metabolite of the original molecule and the third fraction is a more slowly biodegradable metabolite resulting from the secondary degradation. For primary degradation, hydrolysis with no associated growth was assumed. The growth of microorganisms responsible for degradation of the second and third COD fractions was presumed to follow Haldane and first order kinetics, respectively. The model was able to fit four consecutive assays of the same acclimatisation process, using Brij 30 as carbon source, with different food/microorganism ratios. The parameters obtained showed that the (self-)inhibition of the growth on the second COD fraction decreased along acclimatisation.

**Keywords** Acclimatisation; modelling; parameter estimation; respirometry; surfactants

## Introduction

Textile effluents frequently vary in composition and load, depending on the fibre and process being used. This can be a hindrance to their biological treatment. Therefore it is useful to develop ways of describing and predicting the activated sludge response when submitted to fluctuations in the feed. Surfactants currently constitute one of the major chemical oxygen demand (COD) fractions in textile effluents (Knudsen and Wenzel, 1996), and non-ionic surfactants are the most common in textile processing (Patoczka and Pulliam, 1990). Among these, ethylene oxide adducts represent the great majority (Shore, 1990). The biodegradation of polyethoxylated surfactants is known to begin either by the alkane- or alkylphenol-ether bond cleavage or by stepwise oxidation of the ethoxylated chain (Kravetz, 1981; Brunner *et al*., 1988; Ahel *et al.*, 1994; Maki *et al.*, 1994; Tidswell *et al.* 1996). In any case, previous studies suggested that the degradation occurs in multiple steps, probably requiring multiple enzymatic or bacterial consortia (Carvalho *et al.*, in press).

Respirometry is a well established method to obtain activated sludge kinetic information (Spanjers and Vanrolleghem, 1995; Vanrolleghem *et al.*, 1999). It is a very sensitive technique and it represents a powerful tool for assessing the condition of a system, since it is directly related to biomass growth and substrate consumption (Spanjers *et al.*, 1997).

The aim of this work was to find the simplest model that fitted respirometric data corresponding to several steps of an acclimatisation process, keeping structural identifiability (Dochain *et al.*, 1995) and obtaining sensible parameters. However, a simple model rarely can satisfactorily fit experimental data, and the complexity of the processes often requires more elaborate models. Observed saturation and inhibition effects have been introduced by using Monod and Haldane type kinetics. Moreover, a structured model, considering

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different COD compartments, is commonly used for wastewater treatment plants (Henze *et al.*, 1987; Babuna *et al.*, 1998), although it is not usually applied when a single carbon source is used. However, the degradation of the metabolites resulting from primary biodegradation might be equivalent to the one observed for a mixed effluent feed. This was the principle applied in the development of the model presented in this work.

Several optimisation methods are available to fit a model to the measured data, using numerical (indirect) techniques to find parameter values that give the lowest deviation between model predictions and experimental data (Vanrolleghem *et al.*, 1995). In constrast to these, direct methods allow for specific determination of parameters, but they require particular experimental designs, where individual components must be dominant during a critical phase of the respirometric experiment (Spanjers *et al.*, 1999). Therefore, indirect optimisation methods were applied in this work, by means of the Matlab software (Simulink Toolbox, The Mathworks Inc.) and the WEST (Hemmis NV, Kortrijk, Belgium) modelling and simulation software.



## Materials and methods

### Materials

The experimental data were obtained on a bench scale sequencing-batch reactor (SBR), connected to a closed respirometry cell. The volumes of the reactor and cell were 4.8 and 0.8 l, respectively. The 24-hour cycle was computer-controlled and consisted of 25 min drawing off the exhausted supernatant, 17 min idle, 28 min filling, 21h 50 min aeration, and 60 min settling. The periodic pumping of mixed-liquor samples into the respirometric cell and dissolved oxygen measurements were also computer-controlled. A hydraulic retention time of 26 h and a sludge retention time of 15 days were imposed. Air was supplied by an air compressor through ceramic diffusers and additional mixing was provided by magnetic stirring. The average dissolved oxygen concentration was 6.5 $mgO_2/l$, the pH was 7.0±0.2 and the temperature was set at 26±1ºC. Before performing the injection of surfactant (carbon source) to the reactor, at the end of the fill phase, a minimum amount of anti-foam agent (Antifoam A, Sigma,USA) was added (non-metabolised). The sludge used to start up the SBR was taken from an urban wastewater treatment plant (Beirolas, Loures, Portugal) receiving mixed domestic-industrial wastewater. A basic nutrient solution was fed at the beginning of each cycle, excluding the carbon source. It was prepared in aerated tap water and it was composed of NH4Cl (in a proportion of 5 gN: 100 gCOD), phosphate buffer, mineral additive solutions (macro and micro-nutrients), and allylthiourea (ATU, 20 mg/l) as nitrifying inhibitor. All chemicals used were of analytical grade (Merck, Germany; Riedel-de Haën, Germany; Sigma, USA). The carbon source used in the modelled assays was polyethoxylate 4-lauryl ether (Brij 30), a laboratory-grade non-ionic surfactant from Sigma, USA. The sludge used in the acclimatisation assays had been previously adapted to a commercial product (Hostapal SF, non-ionic surfactant mixture containing nonylphenol polyethoxylate) used in textile industry as washing agent (Hoechst Portuguesa, Portugal).

### Analytical techniques

Closed respirometry was used for monitoring the uptake of surfactants by the sludge during the aerated phase. This technique was chosen in order to avoid surfactant interference with the gas-liquid oxygen transfer during measurements. A sample from the reactor mixed liquor was periodically transferred to the closed respirometric cell in which the oxygen consumption rate was directly measured with an oxygen probe. Non-ionic surfactant concentration (NIO) was followed by a titrimetric method based on the formation of $BaCl_2$-surfactant complexes, which were titrated (Titrino 702 SM) with sodium tetraphenylborate, using a specific electrode and a reference Ag/AgCl electrode (Metrohm, Switzerland, Application

Bulletin No.230/1e). This method allows the determination of concentrations of ethoxylated surfactants to a limit of 4 ethoxylene groups (EO). Since the studied surfactant (Brij 30) contains only 4 EO, the values measured by this technique were considered to correspond to the concentration of intact surfactant molecules, before any degradation occurred. COD and total suspended solids (TSS) were determined by standard methods (American Public Health Association, 1995). Total organic carbon (TOC) was measured in a Dhormann (USA) apparatus, model DC-190, according to the manufacturer's instructions. Dissolved oxygen was measured in the respirometric cell with a YSI (USA) Biological Oxygen Monitor, model 5300.



#### Methods

Sludge acclimatisation to Brij 30 was followed along five 24-hour SBR cycles. Surfactant biodegradation was monitored by respirometry and COD, TOC and NIO measurements during the 1st, 2nd, 4th and 5th assays.

In order to check for eventual inhibition effects, initial respiration rate curves with different added substrate concentrations were performed with acclimatised sludge. After washing with phosphate buffer pH 7.0, the sludge was diluted to 1500mgSS/l, ATU (20 mg/l) was added, and the suspension was aerated for about 30 minutes. A 10 ml sample was then taken and placed in a 15 ml closed respirometric cell. The dissolved oxygen concentration was measured for about 10 minutes before carbon source addition for determination of the endogenous respiration rate ($r_{O2\ init.\ (endo.)}$). The surfactant was then added at concentrations ($S_0/X_0$) between 0.05 and 0.5 gCOD(substrate)/gCOD(biomass). The total initial respiration rate ($r_{O2\ init.\ (total)}$) is given by the slope of the dissolved oxygen versus time after injection and it corresponds to the first $r_{O2}$ measurement in a respirogram (plot of the respiration rate versus time). The true initial respiration rate ($r_{O2\ init.}$) was calculated by subtracting the $r_{O2\ init.\ (endo.)}$ from the $r_{O2\ init.\ (total)}$.

#### Mathematical techniques

The parameter estimation procedure consisted of minimising the weighted sum of squared errors (SSE) by using the Brent (1973) direction set optimisation algorithm. The weights used in the fitting multivariable criterium were 1000 and 0.0001 for respirometric and NIO data, respectively.

## Results and discussion

#### Monod based model

As a first attempt, a Monod based model was used to interpret respirometric curves obtained from the injection of Brij 30 to acclimatised sludge. The Matlab/Simulink software was used to simulate the result of the differential equations given a set of parameters and the initial conditions. However, with this model it was not possible to find a combination of parameter values that correctly fitted the initial ascending slope of the respirometric curves, as can be seen by the example shown in Figure 1a.

#### Substrate inhibition based model

It was hypothesised that the initial ascending slope observed in practically all respirograms obtained with surfactants (Carvalho *et al.*, in press) could be the result of substrate inhibition. Nevertheless, the introduction of the Haldane coefficient for inhibition was not enough to properly model the observed data, as depicted in Figure 1b.The variation of the initial sludge respiration rate at a range of $S_0/X_0$ ratios (Figure 2) show that Brij 30 has no apparent inhibitory effect at these concentration levels. This result confirms that the respirogram presented on figure 1 ($S_0/X_0$=0.5 gCOD/gCOD) could not be explained by a

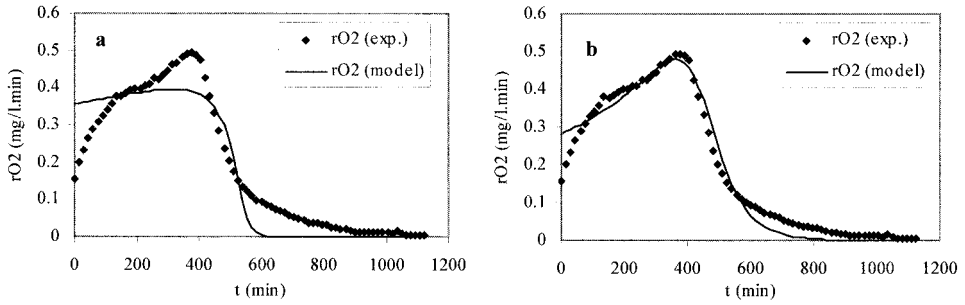


**Figure 1** Respirogram obtained from the injection of Brij 30, with $S_0/X_0$=0.5 gCOD/gCOD, $X_{H1}$=882 mgCOD/l and $S_{s1}$=464 mgCOD/l. (a) Best fitting of the Monod based model to the experimental data was obtained with the following parameter values: $K_{S1}$=20 mg/l; $Y_{H1}$=0.58 mg/mg; ($m_{H1}$=5.8×10$^{-4}$ min$^{-1}$; $b_H$=10$^{-5}$ min$^{-1}$ (see text for parameter definition). Minimum SSE = 0.26. (b) Best fitting of the Haldane based model to the experimental data was obtained with the following parameter values: $K_{S1}$=700 mg/l; $Y_{H1}$=0.55 mg/mg; ($m_{H1}$=0.005 min-1; $b_H$=10$^{-5}$ min$^{-1}$; $K_{I1}$=45 mg/l (see text for parameter definition. $K_{I1}$ is the inhibition constant for $S_{s1}$). Minimum SSE = 0.07

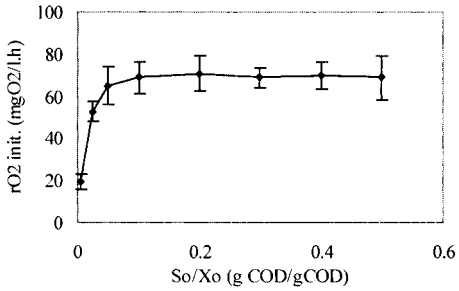


**Figure 2** Initial respiration rate ($r_{O2\,init.}$) of acclimatised sludge for increasing $S_0/X_0$ ratios. $X_0$ was 1500 mgSS/l. Brij 30 concentration was varied from 0.04 to 0.45 g/l

substrate inhibition Haldane kinetics. If any inhibition effect exists, it must be due to a metabolite, since it does not affect the initial respiration rate ($r_{O2\,init.}$).

Furthermore, previous studies revealed that non-acclimatised sludge gives multiple-peak respirograms (such as the one depicted in Figure 3) when submitted to non-ionic surfactants (Carvalho *et al.*, in press), a kind of profile that could not be predicted by a simple Haldane model.

#### Fractionated degradation model

Since the two previous models did not fit the experimental data, a different approach was tried. The surfactant molecule and its metabolites were considered to be degraded by different bacterial or enzymatic consortia, with different associated kinetic parameters. The fact that two respirometric peaks are obtained with non-acclimatised sludge suggests that the second part of degradation is affected by inhibition, which would explain the delay observed in the associated respiration rate. Therefore, it was assumed that the growth upon the uptake of the second COD fraction followed Haldane inhibition kinetics (Eq. (4), below). This model was fitted to both respirometric and surfactant concentration data. The initial measured surfactant concentration was considered to correspond to the first COD fraction ($Ss_1$). Primary degradation was assumed to be mostly enzymatic, with no growth associated to it. Therefore, a hydrolysis equation (Eq. (7), below) of the Michaelis-Menten type was used to describe this mechanism. Assay 1 was considered to start with a very low active biomass concentration, which was allowed to grow mainly during the second

respirometric peak.The starting active biomass concentrations of the following assays were the values obtained at the end of the simulation of the previous one.

Inspired by Vanrolleghem *et al.* (1998), a transition parameter (*Trans*, Eq. (3), below) was introduced in order to justify the initial start-up phenomenon observed in the respiration rate curve. A few minutes are probably needed for solution homogenisation and biomass adaptation after the injection of the substrate. This term was only applied to the equation of growth associated to the second COD fraction, since no growth occurs during the biodegradation of the first fraction.

The applied model fitted well to the experimental data, except for the initial point, which was always too low, and the final tail, for which the model results were always decreasing too fast. The problem of the initial point was solved by using an extra degree of freedom introduced by the initial values which were measured. For time zero, the respiration rate is only dependent on growth upon the second COD fraction (see Eq. (10)). This allows for derivation of an equation to calculate β (Eq. (2), below), a parameter included in the *Trans* term, that can be calculated from initial values at time zero (respiration rate $r_O(0)$, biomass concentration $X_H(0)$ and surfactant concentration $Ss_2(0)$). At $t = 0$,

$$r_O(0) = X_H(0)\left(1 - \beta e^{-t/\tau}\right)\mu_{mH_2} \frac{Ss_2(0)}{K_{S_2} + Ss_2(0) + Ss_2(0)^2 / K_I} \times \frac{1 - Y_{H_2} = Y_{Ss_3}}{Y_{H_2}} \quad (1)$$

where:

$r_O(0)$ = initial exogenous respiration rate (mg/l.min)

$X_H(0)$ = initial active heterotrophic biomass concentration (mgCOD/l)

β = transition parameter

$t$ = assay time after surfactant injection (min)

τ = transition time constant (min)

$\mu_{m_{H2}}$ = maximum specific growth rate for $S_{s2}$ ($min^{-1}$)

$Ss_2(0)$ = initial concentration of surfactant's metabolites (second COD fraction, mgCOD/l). A very low value (approximately 1% of $Ss_1(0)$) was assumed.

$Ks_2$ = heterotrophic affinity constant for $Ss_1$(mgCOD/l)

$K_I$ = inhibition constant for$Ss_2$ (mgCOD/l)

$Y_{Ss_3}$ = yield of conversion of $Ss_2$ into $Ss_3$ (mgCOD/mgCOD)

$Y_{H2}$ = yield of heterotrophic biomass for $Ss_2$ (mgCOD/mgCOD).

The problem of the final part of the respirogram was solved by defining a third much more slowly biodegradable intermediate ($Ss_3$), resulting from the degradation of $Ss_2$. It was assumed that the growth associated to its uptake followed first order kinetics (Eqs. (5) and (9), below).

The final proposed model is based, thus, on the following scheme:

$$Ss_1 \rightarrow Ss_2 \overset{O_2}{\underset{X_{BH}}{\rightleftharpoons}} Ss_3 \overset{O_2}{\underset{X_{BH}}{\rightleftharpoons}}$$

and includes Eqs.(2)–(10).

$$\beta = 1 - \frac{K_{S_2} + Ss_2(0) + Ss_2(0)^2 / K_I}{Ss_2(0)\mu_{mH_2}} \frac{r_O(0)Y_{H_2}}{X_H(0)(1 - Y_{H_2} - Y_{Ss_3})} \quad (2)$$

$$Trans = 1 - \beta e^{-t/\tau} \quad (3)$$

$$\mu_{H_2} = Trans\mu_{mH2}\frac{Ss_2}{K_{S_2} + Ss_2 + Ss_2^2 / K_I} \tag{4}$$

$$\mu_{H3} = k_3 Ss_3 \tag{5}$$

$$\frac{dX_H}{dt} = \left(\mu_{H_2} + \mu_{H_3} - b_H\right)X_H \tag{6}$$

$$\frac{dSs_1}{dt} = -k_h\frac{Ss_1^* X_H}{K_{S_1} + Ss_1} \tag{7}$$

$$\frac{dSs_2}{dt} = -\frac{\mu_{H_2} X_H}{Y_{H_2}} + k_h\frac{Ss_1 X_H}{K_{S_1} + Ss_1} \tag{8}$$

$$\frac{dSs_3}{dt} = -\frac{\mu_{H_3} X_H}{Y_{H_3}} + Yss_3\frac{\mu_{H_2} X_H}{Y_{H_2}} \tag{9}$$

$$r_O = \left[\frac{1 - Y_{H_2} - Y_{Ss_3}}{Y_{H_2}}\mu_{H_2} + \frac{1 - Y_{H_3}}{Y_{H_3}}\mu_{H_3}\right]X_H \tag{10}$$

where:

$\mu_{H_{2,3}}$ = specific growth rates of heterotrophic biomass for $Ss_2$ and $Ss_3$, respectively ($min^{-1}$)
$K_{S1}$ = heterotrophic affinity constant for $S_{s1}$ (mgCOD/l)
$Y_{H3}$ = yield of heterotrophic biomass for $Ss_3$ (mgCOD/mgCOD)
$Ss_1$ = concentration of surfactant in its initial form (first COD fraction, mgCOD/l)
$k_h$ = hydrolysis constant of $Ss_1$ ($min^{-1}$)
$Ss_3$ = concentration of the surfactant's secondary metabolite (third COD fraction, mgCOD/l)
$k_3$ = first order kinetic constant for $Ss_3$ (mgCOD/l.min)
$b_H$ = decay rate of heterotrophic biomass ($min^{-1}$).

This model was tested with several sets of experimental data, corresponding to the sequential assays of an acclimatisation experiment, and good fittings were achieved. Figures 3 to 6 show the fittings obtained with four assays of the acclimatisation to Brij 30. The presented $S_0/X_0$ was calculated with the total biomass concentration. The corresponding obtained parameters are displayed in Table 1.

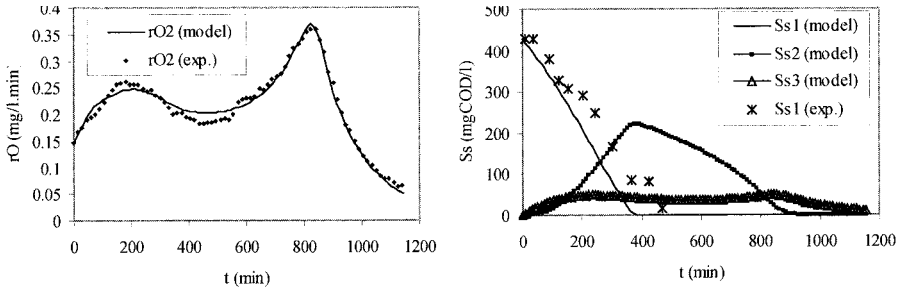


**Figure 3** Experimental data and results predicted by the model for respiration rate and substrates concentrations of the first 24-hour assay of a SBR acclimatisation sequence with Brij 30.
$S_0/X_0$ = 0.5gCOD/gCOD

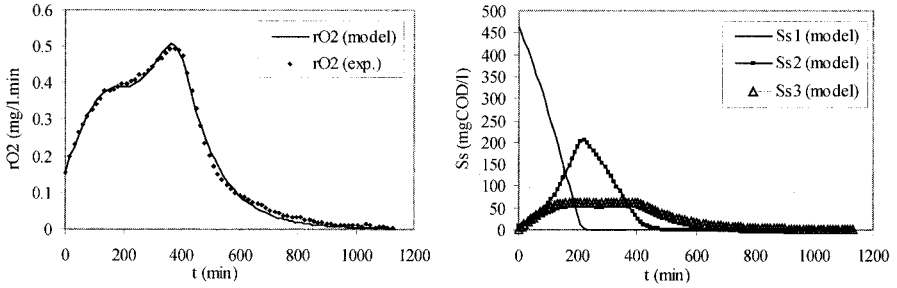


**Figure 4** Experimental data and results predicted by the model for respiration rate and substrates concentrations of the second 24-hour assay of a SBR acclimatisation sequence with Brij 30. $S_0/X_0$ = 0.5gCOD/gCOD

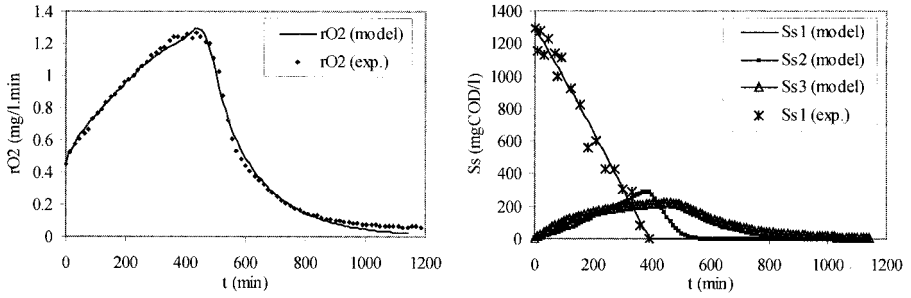


**Figure 5** Experimental data and results predicted by the model for respiration rate and substrates concentrations of the fourth 24-hour assay of a SBR acclimatisation sequence with Brij 30. $S_0/X_0$= 1 gCOD/gCOD

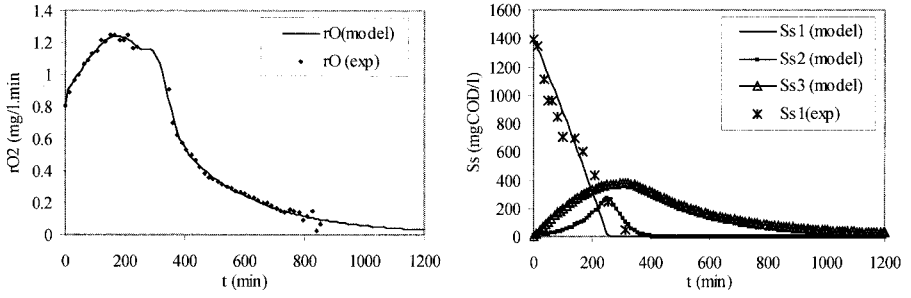


**Figure 6** Experimental data and results predicted by the model for respiration rate and substrates concentrations of the fifth 24-hour assay of a SBR acclimatisation sequence with Brij 30. $S_0/X_0$= 1 gCOD/gCOD

The evolution of the parameters along the four assays revealed the acclimatisation process. The multiple-peak respirograms obtained with non-acclimatised sludge were followed by single peak respirograms, as acclimatisation took place, with shorter degradation times. Apparently, from the model, the main cause for this effect was the loss of inhibition of the secondary degradation step (increase of $K_I$).

#### Statistical comparison of models

The SSE obtained for the four assays, using respirometric and NIO data, are presented in Table 2. From these values it can be concluded that the fractionated degradation model was

**Table 1** Optimal values obtained by fitting the proposed model to the experimental data of Figures 3–6

| Parameters | Assay 1 | Assay 2 | Assay 4 | Assay 5 |
|---|---|---|---|---|
| $r_O(0)$ ($mgO_2$/l.min) | 0.147 | 0.156 | 0.450 | 0.812 |
| $Ss_1(0)$ (mgCOD/l) | 427 | 464 | 1295 | 1391 |
| $Ss_2$ (0) (mgCOD/l) | 0.7 | 8 | 10 | 10 |
| $X_H$ (0) (mgCOD/l) | 80 | 160 | 950 | 950 |
| $K_{S1}$ (mgCOD/l)* | 10 | 10 | 10 | 10 |
| $k_h$ ($min^{-1}$) | $11.0\times10^{-3}$ | $11.0\times10^{-3}$ | $3.23\times10^{-3}$ | $5.06\times10^{-3}$ |
| $\mu_{mH2}$ ($min^{-1}$) | $10.3\times10^{-3}$ | $7.30\times10^{-3}$ | $2.28\times10^{-3}$ | $1.96\times10^{-3}$ |
| $K_{S2}$ (mgCOD/l) | 151 | 150 | 210 | 204 |
| $Y_{H2}$ (mgCOD/mgCOD) | 0.338 | 0.453 | 0.331 | 0.331 |
| $K_I$ (mgCOD/l) | 16.1 | 53.8 | 212 | 216 |
| $b_H$ ($min^{-1}$)* | 5*10-4 | 5*10-4 | 5*10-4 | 5*10-4 |
| $\tau$ (min) | 25.2 | 37.7 | 44.8 | 64.7 |
| $Y_{Ss3}$ (mgCOD/mgCOD) | 0.491 | 0.436 | 0.5 | 0.478 |
| $Y_{H3}$ (mgCOD/mgCOD) | 0.131 | 0.223 | 0.343 | 0.553 |
| $k_3$ (l/mgCOD.min) | $4.45\times10^{-6}$ | $5.30\times10^{-6}$ | $1.64\times10^{-6}$ | $1.44\times10^{-6}$ |

* Parameters fixed when optimising

**Table 2** Minimum sum of squared errors (SSE) obtained for $r_{O2}$ and NIO data (when available) corresponding to the four studied assays of the acclimatisation process

| Model | Assay 1 | | Assay 2 | | Assay 4 | | Assay 5 | |
|---|---|---|---|---|---|---|---|---|
| | $SSE_{rO2}$ | $SSES_{s1}$ | $SSE_{rO2}$ | $SSES_{s1}$ | $SSE_{rO2}$ | $SSES_{s1}$ | $SSE_{rO2}$ | $SSES_{s1}$ |
| Monod | 0.23 | 197,357 | 0.26 | – | 2.1 | 1,157,340 | 1.3 | 1,950,600 |
| Haldane | 0.13 | 262,671 | 0.070 | – | 0.61 | 1,737,830 | 0.82 | 1,932,300 |
| Fractionated degradation | 0.0074 | 8 647 | 0.0079 | – | 0.067 | 80,900 | 0.036 | 651,050 |

significantly better than the other two (Monod and Haldane). For a statistical comparison of the different models (Monod, Haldane and fractionated degradation), F-tests were performed on the SSE.

## Conclusions

A dynamic simulation and automatic optimisation method was used to model an activated sludge acclimatisation to a non-ionic surfactant. The model developed was able to fit experimental data obtained on consecutive SBR cycles of the acclimatisation process. It was possible to use the same model to fit data obtained with different food/microorganism ratios. The model was based on the assumption that different metabolites of the original molecule were degraded by specific enzymatic or bacterial consortia with distinct degradation rates. Three consecutive COD fractions were considered, associated with different kinetic profiles. A significantly better fit was obtained with the fractionated degradation model than with other simpler models (Monod and Haldane).

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